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Lsm Proteins Promote Regeneration of Pre-mRNA Splicing Activity

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Summary

Lsm proteins are ubiquitous, multifunctional proteins that affect the processing of most RNAs in eukaryotic cells [1–11], but their function is unknown. A complex of seven Lsm proteins, Lsm2-8, associates with the U6 small nuclear RNA (snRNA) that is a component of spliceosome complexes in which pre-mRNA splicing occurs. Spliceosomes contain five snRNAs, U1, U2, U4, U5, and U6, that are packaged as ribonucleoprotein particles (snRNPs) [12, 13]. U4 and U6 snRNAs contain extensive sequence complementarity and interact to form U4/U6 di-snRNPs. U4/U6 di-snRNPs associate with U5 snRNPs to form U4/U6.U5 tri-snRNPs prior to spliceosome assembly. Within spliceosomes, disruption of base-paired U4/U6 heterodimer allows U6 snRNA to form part of the catalytic center [14]. Following completion of the splicing reaction, snRNPs must be recycled for subsequent rounds of splicing, although little is known about this process. Here we present evidence that regeneration of splicing activity in vitro is dependent on Lsm proteins. RNP reconstitution experiments with exogenous U6 RNA show that Lsm proteins promote the formation of U6-containing complexes and suggest that Lsm proteins have a chaperone-like function, supporting the assembly or remodeling of RNP complexes involved in splicing. Such a function could explain the involvement of Lsm proteins in a wide variety of RNA processing pathways.

Results and Discussion

Lsm Proteins Promote Regeneration of Splicing Activity

In *Saccharomyces cerevisiae*, *LSM6* and *LSM7* are nonessential genes, but their deletion causes heat sensitivity and a mild splicing defect [1, 2]. Here, we show that although extracts from *lsm6*Δ or *lsm7*Δ strains are competent to splice *ACT1* pre-mRNA (Figure 1, lanes 3 and 7, and data not shown), they fail to support splicing during sequential incubations. Extracts were first incubated with an excess (35 nM) of unlabeled *ACT1* pre-mRNA under splicing conditions, followed by the addition of a small amount (2 nM) of ^{32}P-labeled *ACT1* pre-mRNA for a second incubation. Unlike wild-type extract, which was able to support splicing during multiple sequential incubations (Figure 1, lane 2, and data not shown), *lsm6*Δ and *lsm7*Δ extracts could not support splicing during a second incubation with pre-mRNA (Figure 1, lanes 4 and 8). Significantly, this loss of splicing activity was splicing dependent and not simply due to incubation of the extracts (Figure 1, lanes 1, 3, and 7), nor was it due to depletion of ATP (data not shown). Following the incubation of *lsm6*Δ or *lsm7*Δ extracts with unlabeled pre-mRNA, the addition of the missing Lsm protein, produced by in vitro translation, made these extracts competent for splicing during a second incubation (Figure 1, lanes 5 and 10). This effect was Lsm protein specific; Lsm7p did not restore activity to Lsm6p-depleted extract and vice versa (Figure 1, lanes 6 and 9). As the exogenous proteins were added after the first incubation with substrate RNA, we conclude that a factor (or factors) became limiting in a splicing-dependent manner in the absence of Lsm6p or Lsm7p, but could be regenerated by the addition of the missing Lsm protein.

Lsm Proteins Are Required for U6 RNP Reconstitution

Lsm2-8 proteins associate directly with U6 snRNA [3, 15] and are required for its stability [1]. The analysis of *lsm* mutant extracts is therefore complicated by their reduced level of U6 snRNA. To obtain conditions in which the level of U6 RNA is initially equivalent in the different extracts, we ablated the endogenous U6 snRNA by targeted RNase H digestion, added in vitro transcribed U6 RNA to each extract to 50 nM, and assayed splicing activity and the fate of the exogenous U6 RNA. Following the ablation of endogenous U6 snRNA (Figure 2, lanes 2, 5, and 9), exogenous U6 RNA very efficiently restored the splicing activity of wild-type but not of the mutant extracts (Figure 2, lanes 3, 6, and 10). This defect in *lsm6*Δ and *lsm7*Δ mutant extracts could be at least partly overcome by the addition of the appropriate Lsm protein produced in vitro (Figure 2, lanes 7 and 11). Thus, in the absence of Lsm6p or Lsm7p, exogenous U6 RNA could not replace the ablated U6 snRNA in the splicing process.

To investigate the association of the exogenous U6 RNA with the endogenous U4 snRNA, total RNA from mutant and wild-type extracts was deproteinized and analyzed by nondenaturing gel electrophoresis. As reported previously [1], the level of U6 snRNA was lower in the unablated mutant extracts compared to the wild-type extract (Figure 3A, left panel, lanes 1, 4, and 8), and most of the U6 snRNA in the mutant extracts was complexed with U4 snRNA. Following ablation of the endogenous U6 snRNA in wild-type extract, 76% of the U4 snRNA became complexed with exogenous U6 RNA

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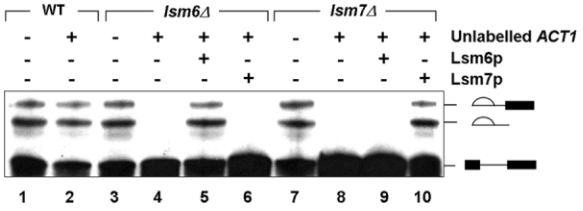

Figure 1. Regenerating Splicing Activity Requires Lsm Proteins

Whole-cell extracts (4 μl) prepared from wild-type, *lsm6*Δ, or *lsm7*Δ strains were incubated at 23°C under in vitro splicing conditions either in the absence (lanes 1, 3, and 7) or presence (lanes 2, 4–6, and 8–10) of 35 nM unlabeled *ACT1* pre-mRNA as indicated for 30 min. 2 μl of in vitro translation reaction, containing Lsm6p (lanes 5 and 9) or Lsm7p (lanes 6 and 10) or mock reaction (lanes 1–4 and 7–8), were then added as indicated and all samples were incubated with 2 nM ^{32}P-labeled *ACT1* pre-mRNA for 30 min. RNA was then extracted and analyzed on a 7% polyacrylamide-urea gel and visualized by autoradiography.

(Figure 3A, right panel, lane 3), whereas only 26% and 51% of U4 snRNA became incorporated into U4/U6 heterodimer in the *lsm6*Δ and *lsm7*Δ mutant extracts, respectively (Figure 3A, right panel, lanes 6 and 10). With the addition of in vitro produced Lsm6p to *lsm6*Δ extract or Lsm7p to *lsm7*Δ extract, the association of U4 with U6 RNA increased to 58% and 60%, respectively (Figure 3A, right panel, lanes 7 and 11). Thus, in the absence of a complete set of Lsm proteins, the efficiency of U4/U6 formation was reduced, with the residual activity presumably being promoted by Prp24p, another U6-associated protein, that facilitates the annealing of the U4 and U6 snRNAs [16, 17]. However, U4/U6 duplex formation was more efficient when the missing Lsm protein was added. Note that the level of U4/U6 is a balance of U4/U6 annealing and dissociation, and this assay does not distinguish between a requirement for Lsm proteins for annealing of U4 with U6 or for stabilization of the U4/U6 duplex; however, Achsel et al. [3] showed that isolated human LSm2-8 complex promoted the formation of U4/U6 RNP particles in vitro.

Also, in the *lsm6*Δ extract, the total amount of U6 RNA was 60% higher in the presence compared to the absence of Lsm6p (comparing lanes 6 and 7 in Figure

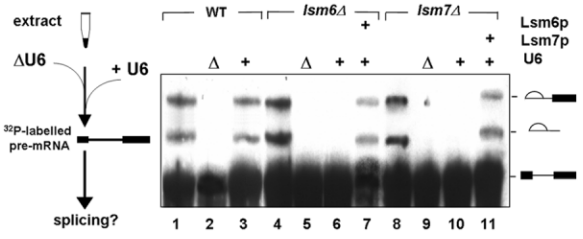

Figure 2. Reconstitution of Splicing with Exogenous U6 RNA In Vitro and Analysis of Splicing Activity

Lanes 1, 4, and 8 show splicing activity with 4 μl untreated wild-type, *lsm6*Δ, and *lsm7*Δ extracts, respectively. For all other lanes, extracts were incubated with 140 nM U6 knockout oligo. For lanes 3, 6, 7, 10, and 11, in vitro transcribed U6 RNA was added (to 50 nM) to the ablated samples. 2 μl of in vitro translation reaction containing Lsm6p or Lsm7p were added as indicated, and splicing activity was assayed using 2 nM ^{32}P-labeled *ACT1* pre-mRNA.

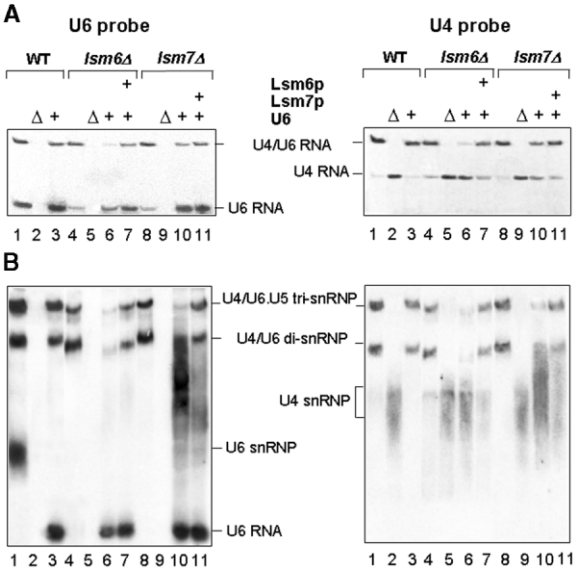

Figure 3. Analysis of the Incorporation of U6 RNA into Complexes

(A) Annealing of exogenous U6 RNA with endogenous U4 snRNA. RNA was extracted from extracts (4 μl) that had been treated as in Figure 2 but without ACT1 pre-mRNA, and the level of U4/U6 heterodimer was analyzed by nondenaturing gel electrophoresis and Northern blotting, probing for U6 RNA (left) or U4 RNA (right). Note that the level of both endogenous and exogenous U6 RNA in *lsm6*Δ extract was slightly lower due to degradation while incubating in the absence of Lsm6p.

(B) Incorporation of exogenous U6 RNA into snRNP complexes. RNP complexes from extracts (4 μl), treated as in (A) were resolved by nondenaturing gel electrophoresis, electroblotted, and probed for U6 snRNA (left) or U4 snRNA (right).

3A, left panel). Thus, Lsm6p appeared to stabilize the U6 RNA during the incubation, whereas there was little or no effect of Lsm7p on the total U6 RNA in *lsm7*Δ extract. This stabilization of U6 RNA complicates the interpretation of the effect of the Lsm6p on the level of U4/U6 dimer, as a higher level of U6 RNA might drive the equilibrium toward U4/U6 formation. Thus, we conclude that Lsm6p is required for maintenance of U6 RNA and directly or indirectly affects the proportion of U4 snRNA that is complexed with U6 RNA. Lsm7p has a smaller, but nevertheless significant, effect on the level of U4/U6 dimer in the absence of a significant effect on the level of total U6 RNA.

Next, the assembly of the exogenous U6 RNA into RNP particles was examined by native gel electrophoresis. Ablation of the U6 snRNA caused the U4 snRNA to migrate as a heterogeneous smear (Figure 3B, right panel, lanes 2, 5, and 9). This smear represents a mixture of heterogeneous U4 particles that result from ablation of the U6 snRNA that was in di- and tri-snRNPs. Following the addition of exogenous U6 RNA, no free U6 snRNPs were detectable, even in the wild-type extract; however, all of the U4 RNA in wild-type extract was incorporated into di-snRNPs and tri-snRNPs (Figure 3B, lanes 3). In *lsm7*Δ extract, the exogenous U6 RNA was incorporated into a smear of RNP complexes that also contained U4 snRNA (Figure 3B, lane 10). However, these migrated faster than the wild-type U4/U6 di-

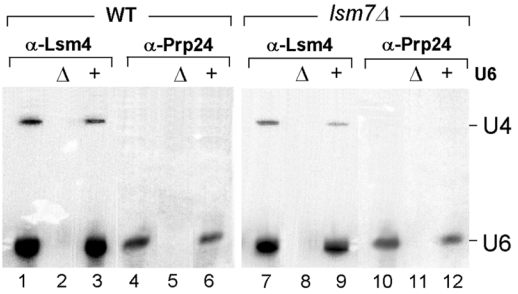

Figure 4. Coprecipitation of U4 and U6 RNAs with Lsm4p and Prp24p

Extracts from wild-type or *lsm7*Δ cells were either left untreated (lanes 1, 4, 7, and 10) or were incubated with U6 knockout oligo to ablate U6 snRNA (all other lanes). For lanes 3, 6, 9, and 12, in vitro transcribed U6 RNA was added to 50 nM. Samples were incubated with either anti-Lsm4 (lanes 1–3 and 7–9) or anti-Prp24 (lanes 4–6 and 10–12) antibodies. RNA was extracted from precipitates, fractionated by denaturing gel electrophoresis, and analyzed by Northern blotting with probes for U4 and U6 RNAs.

snRNPs, indicating that they are not correctly or fully assembled, and very little U6 or U4 RNA was found in tri-snRNP complex. Addition of in vitro translated Lsm7p shifted most of the heterogeneous U4/U6 RNPs into complexes that comigrated with endogenous di-snRNP and tri-snRNP complexes (Figures 4, lane 11). With *lsm6*Δ extract, a similar result was obtained, although the more significant effect of Lsm6p was on the level of U6 RNA (Figures 3B, lanes 6 and 7). Therefore, in the absence of Lsm7p, although a significant amount (51%) of U4 snRNA was in the form of U4/U6 heterodimer, di-snRNP formation was defective and little or no tri-snRNP formation occurred.

These results suggest that the inability of extracts that lack either Lsm6p or Lsm7p to support splicing through multiple incubations is most likely due to the inability to reassemble functional U4/U6 and U4/U6.U5 snRNP complexes that were depleted during the splicing process. Although the effects of Lsm6p and Lsm7p differ in the greater need for Lsm6p for U6 RNA stability, it is not clear at which stage the U6 RNA becomes unstable in the absence of Lsm6p. The primary defect could be the inability to incorporate U6 RNA into snRNP complexes with the subsequent degradation of U6 RNA. Thus, U6 snRNA that is released from dissociating spliceosomes may be degraded in the absence of a complete Lsm protein complex, especially in the absence of Lsm6p.

This defect in the incorporation of exogenous U6 RNA into di- and tri-snRNPs, seen most clearly in *lsm7*Δ cell extracts, could have a number of causes. As the reconstituted U6 RNP complexes had a heterogeneous electrophoretic distribution, other proteins in addition to Lsm7p (or Lsm6p) may be missing from some or all of the complexes. Possibly di-snRNP- or tri-snRNP-specific proteins fail to associate with the Lsm-depleted U4/U6 particle, preventing its conversion to tri-snRNP. Nottrott et al. [18] reported that the association of human U4/U6-specific proteins with preannealed U4/U6 RNA occurred in the absence of Lsm proteins in vitro. Therefore, it may be that some di- and tri-snRNPs formed (indeed, a low level of tri-snRNP was detected) but that these were unstable in cell extract.

The question remains as to what type of complex forms on the U6 snRNA in the absence of an Lsm protein. Immunoprecipitation experiments (Figure 4) showed that in mutant extracts the endogenous U6 snRNA associates with Prp24p, and both U6 and U4 associate with Lsm4p (lanes 7 and 10; also for *lsm6*Δ, data not shown), indicating that a full complement of Lsm proteins is not essential for the binding of these proteins. Following U6 reconstitution, the exogenous U6 RNA also coimmunoprecipitates with Lsm4p and Prp24p (Figure 4, lanes 3, 6, 9, and 12). Therefore, the failure to reconstitute normal di- and tri-snRNPs was not due to a failure to associate with Prp24p or Lsm4p. The very heterogenous nature of the U6-containing RNP complexes in mutant extracts makes their further analysis impractical.

Why Are Lsm6p and Lsm7p Not Essential for Cell Viability?

In view of these important functions for Lsm6p and Lsm7p, it seems surprising that *LSM6* and *LSM7* genes are not essential for cell growth; however, the U6 assembly defect may not be as severe in vivo as it is in vitro. Also, the traditional view of the spliceosome cycle has been challenged by Stevens et al. [19], who isolated a yeast penta-snRNP complex that, when supplied with soluble factors, spliced pre-mRNA. Thus, the penta-snRNP complex may offer an alternative method for regenerating snRNPs. Conceivably, Lsm proteins may only be required to reassemble U6-containing RNPs following the dissociation of multi-snRNP complexes such as penta-snRNPs.

Nevertheless, in *lsm6*Δ and *lsm7*Δ cells, how does newly synthesized U6 RNA get assembled into functional RNP complexes in the first place? It may be speculated that newly transcribed U6 snRNA can be assembled into RNP particles by a different process than U6 snRNA that has been through a splicing reaction. An obvious candidate as a cofactor for assembling nascent U6 snRNA into RNP particles is La protein (Lhp1 in yeast), which has been proposed to act as a chaperone for polymerase III nascent transcripts. A deletion of the *LHP1* gene is synthetic lethal with an *lsm8* mutation, an indicator of genes involved in the same or alternative pathways [20]. Thus, newly transcribed U6 snRNA may assemble into snRNP complexes in an alternative pathway that bypasses the need for Lsm6p or Lsm7p, whereas U6 snRNA in postsplicing complexes (or added to extracts in snRNP reconstitution experiments) would be at least partially dependent on Lsm proteins for the reactivation process.

The related Sm proteins have been proposed to stabilize weak snRNA-pre-mRNA interactions in the spliceosome [21], and the bacterial Sm-like protein, Hfq, also mediates multiple RNA-RNA interactions, suggesting a role as an RNA chaperone [22]. Our data support a chaperone-like role for Lsm proteins in assembling or remodeling RNA-protein complexes during the splicing process. As expected for chaperones, the associations of Lsm proteins with most RNA substrates are highly transient; for example, Lsm1-7 proteins associate with

mRNAs in the cytoplasm [4, 5] and Lsm2-8 proteins associate with pre-tRNAs, pre-rRNAs, and pre-snoRNAs in the nucleus [6–9]. The persistent association of Lsm2-8 complex with U6 snRNA is an exception; however, Chan et al. [23] presented evidence that Lsm proteins dissociate from U6 snRNA in spliceosomes at around the time of spliceosome activation. Thus, Lsm2-8p seem likely to function as a chaperone complex to support multiple rearrangements of U6-containing complexes, including U4/U6 annealing/stabilization, di- and tri-snRNP formation, incorporation, and rearrangement in spliceosomes, at which point their role is complete and they dissociate until required again to regenerate U6 snRNPs. This suggests a general role for Lsm proteins in promoting the assembly or rearrangement of RNP complexes in the many RNA processing pathways in which they are involved. Prp24p was also proposed to play a role in the recycling of U6 snRNPs [17, 24, 25], but the conclusion that Lsm proteins are required in extracts that contain Prp24p indicates that Lsm2-8 complex and Prp24p have nonredundant functions. The different, and possibly cooperative, functions of the Lsm and Prp24 proteins is a focus of our ongoing experiments.

Experimental Procedures

Yeast Strains

Wild-type: BMA38α (*MATα, trpΔ1, his3-Δ200, ura3-1, leu2-3,-112, ade2-1, can1-100*); *lsm6Δ*: AEMY19 (*MATα, trpΔ1, his3-Δ200, ura3-1, leu2-3,-112, ade2-1, can1-100 lsm6Δ:: HIS3*); and *lsm7Δ*: AEMY22 (*MATα, trpΔ1, his3-Δ200, ura3-1, leu2-3,-112, ade2-1, can1-100 lsm7Δ:: HIS3*) [1].

Splicing Assays

Yeast whole-cell extracts were prepared and assayed for splicing activity as [28], using *ACT1* substrate RNA produced in vitro with T7 RNA polymerase and p283 as template [26]. U6 snRNP reconstitution was performed ([29], as modified [15]) using 140 nM U6 knockout oligo and adding in vitro transcribed U6 RNA (pT7U6 as template) to 50 nM.

In Vitro Translation to Produce Lsm6p and Lsm7p

Linear DNA templates for in vitro translation of Lsm6 or Lsm7 protein were produced by PCR using plasmids pAEM34 or pAEM59, respectively [27]. Oligonucleotides 5′LSM6TNT (ACGGATCGTAATACG ACTCACTATAGGGAGATGTCCGGAAAAGCTTCT) and 5′LSM7TNT (ACGGATCGTAATACGACTCACTATAGGGAGATGCATCAGCAA CAC) were the upstream primers for *LSM6* and *LSM7*, respectively, introducing a T7 promoter upstream of the coding sequence. Oligonucleotide LexRev (TTTTAAAACCTAAGAGTCAC) was the downstream primer in both cases. The resulting DNA was gel purified and expressed in a wheat-germ in vitro TNT transcription/translation system (Promega) according to manufacturer's instructions.

RNA and RNP Analyses

RNP

Yeast splicing extract (4 μl) was fractionated in a 4% (w/v) polyacrylamide (80:1 acrylamide:bisacrylamide) nondenaturing gel (70 V for 13 hr at 4°C in 50 mM Tris-borate [pH 8.3], 1 mM EDTA), electroblotted to Hybond-N membrane, and hybridized with oligo probes for U4 and U6 RNA.

RNA

For analysis of U4/U6 heterodimers, RNA was purified from 4 μl of yeast splicing extract by treatment with proteinase K (40 mM Tris-HCl [pH 7.5]; 140 mM NaCl; 1% SDS and 2 μg/μl proteinase K) followed by two extractions with phenol-chloroform and precipitation with ethanol. After fractionation in a 9% (w/v) polyacrylamide nondenaturing gel (37.5:1 acrylamide:bisacrylamide) at 4°C, the RNA was electroblotted to Hybond-N membrane and hybridized with oligo probes for U4 and U6 RNA. Band intensities were quantified using a PhosphorImager.

Immunoprecipitation

Anti-Lsm4 or anti-Prp24 antibodies [15] were bound to protein A-Sepharose beads (PAS, Sigma) in NTN buffer (150 mM NaCl, 50 mM Tris-HCl [pH 7.5], 0.1% Nonidet P-40). PAS bound antibodies were incubated with samples at 4°C for 2 hr; the antibody complexes were washed 4 times with NTN and once with NT (NTN without nonidet P-40). Precipitated RNAs were deproteinized, separated on a 6% polyacrylamide, 8 M urea gel, and analyzed by Northern blotting.

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References

1. Mayes, A.E., Verdone, L., Legrain, P., and Beggs, J.D. (1999). Characterization of Sm-like proteins in yeast and their association with U6 snRNA. EMBO J. *18*, 4321–4331.
2. Salgado-Garrido, J., Bragado-Nilsson, E., Kandels-Lewis, S., and Séraphin, B. (1999). Sm and Sm-like proteins assemble in two related complexes of deep evolutionary origin. EMBO J. *18*, 3451–3462.
3. Achsel, T., Brahms, H., Kastner, B., Bachi, A., Wilm, M., and Lührmann, R. (1999). A doughnut-shaped heteromer of human Sm-like proteins binds to the 3′-end of U6 snRNA, thereby facilitating U4/U6 duplex formation in vitro. EMBO J. *18*, 5789–5802.
4. Tharun, S., He, W., Mayes, A.E., Lennertz, P., Beggs, J.D., and Parker, R. (2000). Yeast Sm-like proteins function in mRNA decapping and decay. Nature *404*, 515–518.
5. Bouveret, E., Rigaut, G., Shevchenko, A., Wilm, M., and Séraphin, B. (2000). An Sm-like protein complex that participates in mRNA degradation. EMBO J. *19*, 1661–1671.
6. Kufel, J., Allmang, C., Verdone, L., Beggs, J.D., and Tollervey, D. (2002). Lsm proteins are required for normal processing of pre-tRNAs and their efficient association with La-homologous protein Lhp1p. Mol. Cell. Biol. *22*, 5248–5256.
7. Kufel, J., and Tollervey, D. (2003). 3′-processing of yeast tRNATrp precedes 5′-processing. RNA *9*, 202–208.
8. Kufel, J., Allmang, C., Petfalski, E., Beggs, J., and Tollervey, D. (2003). Lsm proteins are required for normal processing and stability of ribosomal RNAs. J. Biol. Chem. *278*, 2147–2156.
9. Kufel, J., Allmang, C., Verdone, L., Beggs, J., and Tollervey, D. (2003). A complex pathway for 3′processing of the yeast U3 snoRNA. Nucleic Acids Res. *31*, 6788–6797.
10. Fernandez, C.F., Pannone, B.K., Chen, X., Fuchs, G., and Wolin, S.L. (2004). An Lsm2-Lsm7 complex in *Saccharomyces cerevisiae* associates with the small nucleolar RNA snR5. Mol. Cell. Biol. *15*, 2842–2852.
11. Tomasevic, N., and Peculis, B.A. (2002). *Xenopus* LSm proteins bind U8 snoRNA via an internal evolutionarily conserved octamer sequence. Mol. Cell. Biol. *22*, 4101–4112.
12. Burge, C.B., Tuschl, T., and Sharp, P.A. (1999). Splicing of precursors to mRNAs by the spliceosome. In The RNA World, edition 2, R.F. Gesteland, T.R. Cech, and J.F. Atkins, ed. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press), pp. 525–560.
13. Will, C.L., and Lührmann, R. (2001). Spliceosomal UsnRNP biogenesis, structure and function. Curr. Opin. Cell Biol. *13*, 290–301.

14. Staley, J.P., and Guthrie, C. (1998). Mechanical devices of the spliceosome: motors, clocks, springs, and things. Cell *92*, 315–326.
15. Vidal, V.P., Verdone, L., Mayes, A.E., and Beggs, J.D. (1999). Characterization of U6 snRNA-protein interactions. RNA *5*, 1470–1481.
16. Ghetti, A., Company, M., and Abelson, J. (1995). Specificity of Prp24 binding to RNA: a role for Prp24 in the dynamic interaction of U4 and U6 RNAs. RNA *1*, 132–145.
17. Raghunathan, P.L., and Guthrie, C. (1998). A spliceosomal recycling factor that reanneals U4 and U6 small nuclear ribonucleoprotein particles. Science *279*, 857–860.
18. Nottrott, S., Urlaub, H., and Luhrmann, R. (2002). Hierarchical, clustered protein interactions with U4/U6 snRNA: a biochemical role for U4/U6 proteins. EMBO J. *21*, 5527–5538.
19. Stevens, S.W., Ryan, D.E., Ge, H.Y., Moore, R.E., Young, M.K., Lee, T.D., and Abelson, J. (2002). Composition and functional characterization of the yeast spliceosomal penta-snRNP. Mol. Cell *9*, 31–44.
20. Pannone, B.K., Xue, D., and Wolin, S.L. (1998). A role for the yeast La protein in U6 snRNP assembly: evidence that the La protein is a molecular chaperone for RNA polymerase III transcripts. EMBO J. *17*, 7442–7453.
21. Zhang, D., Abovich, N., and Rosbash, M. (2001). A biochemical function for the Sm complex. Mol. Cell *7*, 319–329.
22. Valentin-Hansen, P., Eriksen, M., and Udesen, C. (2004). The bacterial Sm-like protein Hfq: a key player in RNA transactions. Mol. Microbiol. *51*, 1525–1533.
23. Chan, S.P., Kao, D.I., Tsai, W.Y., and Cheng, S.C. (2003). The Prp19p-associated complex in spliceosome activation. Science *302*, 279–282.
24. Bell, M., Schreiner, S., Damianov, A., Reddy, R., and Bindereif, A. (2002). p110, a novel human U6 snRNP protein and U4/U6 snRNP recycling factor. EMBO J. *21*, 2724–2735.
25. Rader, S.D., and Guthrie, C. (2002). A conserved Lsm-interaction motif in Prp24 required for efficient U4/U6 di-snRNP formation. RNA *8*, 1378–1392.
26. O'Keefe, R.T., Norman, C., and Newman, A.J. (1996). The invariant U5 snRNA loop 1 sequence is dispensable for the first catalytic step of pre-mRNA splicing in yeast. Cell *86*, 679–689.
27. Fromont-Racine, M., Mayes, A.E., Brunet-Simon, A., Rain, J.-C., Coley, A., Dix, I., Decourty, L., Joly, N., Ricard, F., Beggs, J.D., et al. (2000). Genome-wide protein interaction screens reveal functional networks involving Sm-like proteins. Yeast *17*, 95–110.
28. Lin, R.-J., Newman, A.J., Cheng, S.-C., and Abelson, J. (1985). Yeast mRNA splicing in vitro. J. Biol. Chem. *260*, 14780–14792.
29. Fabrizio, P., Mcpheeters, D.S., and Abelson, J. (1989). In vitro assembly of yeast U6 snRNP: a functional assay. Genes Dev. *3*, 2137–2150.